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Comparative transcriptomics of *Pleurotus eryngii* reveals blue-light regulation of carbohydrate-active enzymes (CAZymes) expression at primordium differentiated into fruiting body stage

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ABSTRACT

Blue light is an important environmental factor which could induce mushroom primordium differentiation and fruiting body development. However, the mechanisms of *Pleurotus eryngii* primordium differentiation and development induced by blue light are still unclear. The CAZymes (carbohydrate-active enzymes) play important roles in degradation of renewable lignocelluloses to provide carbohydrates for fungal growth, development and reproduction. In the present research, the expression profiles of genes were measured by comparison between the *Pleurotus eryngii* at primordium differentiated into fruiting body stage after blue light stimulation and dark using high-throughput sequencing approach. After assembly and compared to the *Pleurotus eryngii* reference genome, 11,343 unigenes were identified. 539 differentially expressed genes including white collar 2 type of transcription factor gene, A mating type protein gene, MAP kinase gene, oxidative phosphorylation associated genes, CAZymes genes and other metabolism related genes were identified during primordium differentiated into fruiting body stage after blue light stimulation. KEGG results showed that carbon metabolism, glycolysis/gluconeogenesis and biosynthesis of amino acids pathways were affected during blue light inducing primordia formation. Most importantly, 319 differentially expressed CAZymes participated in carbon metabolism were identified. The expression patterns of six representative CAZymes and laccase genes were further confirmed by qRT-PCR. Enzyme activity results indicated that the activities of CAZymes and laccase were affected in primordium differentiated into fruiting body under blue light stimulation. In conclusion, the comprehensive transcriptome and CAZymes of *Pleurotus eryngii* at primordium differentiated into fruiting body stage after blue light stimulation were obtained. The biological insights gained from this integrative system represent a valuable resource for future genomic studies on this commercially important mushroom.

1. Introduction

The mushrooms development could be divided into three different stages, including hyphal knot, primordium and fruiting body [1]. The primordium differentiated and fruiting body formation is focused as a popular topic in mycological study, which has been illustrated in several model species such as *Coprinopsis cinerea* [2,3], *Schizophyllum commune* [4], *Agaricus bisporus* [5,6], *Boletus edulis* [7] and *Flammulina velutipes* [8,9]. The process of primordium differentiated and fruiting body formation in life cycle of mushrooms is mediated by cellular processes, genetic, physiological and environmental factors. Several genes which participate in this process have been demonstrated previously. For instance, ubiquitin–proteasome, cytochrome P450, hydrophobin, *Noxs* [10], *dst* [11,12], *Ubc2* [13] and *eln2* [14] showed different expression among primordium differentiated and fruiting body formation. In addition, genes participated in MAPK, cAMP and ROS signals were found differentially expressed during these stages [13,15].

Beside the genetic factor, the environmental factor such as light could also affect fruiting body differentiation [16,17]. Several researches showed that blue light could induce differentiation and development of fruiting body in *Hypsizygus marmoreus* [18], *Pleurotus ostreatus* and *Coprinus cinereus* [11,19].Several blue light receptor were found and cloned successfully. The *WC-1/2* of *Schizophyllum commune* [5], *dst1* and *dst2* genes in *Coprinus cinereus* [11,12], *phrA* and *phrB* from *Lentinula edodes* [20,21], *Cmwc-1* in different strains of *Cordyceps*

Abbreviations: AAs, auxiliary activities; CDS, coding DNA sequence; CEs, carbohydrate esterases; CBMs, carbohydrate-binding modules; COG, Cluster of Orthologous Groups; DEGs, differentially expressed genes; FDR, false discovery rate; FPKM, Fragments Per Kilobase of transcript per Million; GO, Gene Ontology; GHs, glycosyl hydrolases; GTs, glycosyl transferases; KEGG, Kyoto Encyclopedia of Genes and Genomes; MnP, manganese peroxidase; NR, non-redundant; PLs, polysaccharide lyases; RH, relative humidity; SRA, Sequence Read Archive

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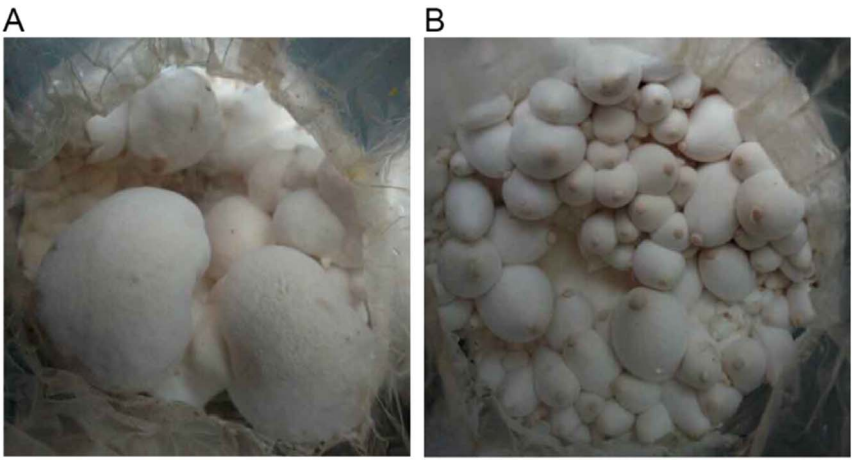

Fig. 1. Primordium differentiated into fruiting body of *P. eryngii* by comparison between the blue light group and the dark group. Dark control (a) and blue light stimulation (b).

militaris [22], and *Slwc-1* from *Sparassis latifolia* were encoded as putative photoreceptor for blue light [23]. In addition to the blue light receptor, it is also found that primary metabolic pathways in oyster mushroom mycelia were regulated induced by blue light stimulation [24]. However, the molecular mechanism of primordium differentiated and fruiting body formation induced by blue light is limited in other non-model commercial mushrooms.

Carbohydrate-active enzymes (also known as CAZy enzymes or CAZymes) are involved in the hydrolysis of plant cell wall polysaccharides, and play an important role in substrate degradation processes [25]. The carbohydrates from degraded lignocellulosic substrate, such as mono and oligosaccharides, can be utilized as nutrition for fungal development and reproduction. Previous research showed that CAZymes play important roles on the sclerotial formation of *Wolfiporia cocos* [26]. In addition, cellulase gene expressions of *Trichoderma reesei* were modulated by blue light. However, it remains unclear whether other CAZymes are involved in fruiting body differentiation and development induced by blue light.

Pleurotus eryngii (*P. eryngii*) is an edible and medicinal white-rot fungus which has been planted extensive in the Mediterranean, central Europe, central Asia, and north Africa due to its remarkable flavor, high nutritional value and numerous medicinal features [27]. In factory production, blue light is used to stimulate fruiting body development in *P. eryngii*. However, the mechanisms of *P. eryngii* primordium differentiation into fruiting body induced by blue light are still unclear. For better understanding the blue light photoresponse on the primordium development in *P. eryngii*, the transcriptomes under dark or blue light were examined. The undifferentiated fruiting body or differentiated fruiting body was measuring and the RNA-seq was performed by using Illumina technology, resulting in the identification of differentially expressed genes. Our previous research showed that *P. eryngii* can also produce various CAZymes to transform all components of plant biomass, including cellulose, hemicellulose, and lignin [28,29]. However, it is still unclear whether blue light induces the expression of CAZymes in primordium differentiated into fruiting body stage. So the expression level and enzyme activity of CAZymes during fruiting body development were also discussed. qRT-PCR was also used to confirm the expression profiles of the CAZymes genes. This transcriptomic information could promote our understanding of the genetic and molecular mechanisms of the development of fruiting body under blue light.

2. Materials and methods

2.1. P. eryngii cultivation

The *P. eryngii* (CICC50126) was obtained from the inquiry network for microbial strains of China. Ramie stalks from Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences were collected and used as main component of substrates. *P. eryngii* was cultured in solid medium containing 50% mixture of ramie stalks, 21% cottonseed hulls, 21% wheat bran, 6% corn meal, 1% sucrose and 1% calcium carbonate. The ratio of material to water was 1:1.5. The mixed substrates were packed in mushroom culture packages, sterilized, and cooled to room temperature. Pre-cultured *P. eryngii* was inoculated onto the top of the substrates in culture packages. To obtain a uniform spread of the hypha in the substrates, packages were kept at 20–24 °C, 65–70% relative humidity (RH) in the dark. When the mycelium colonized the substrate completely within 40 days, the culture packages were moved to an environment at 10–15 °C, 90–95% RH. Bags were then either exposed to a 12 h/day or dark regime with blue light LED illumination (455 nm, 80 μmol m^{-2} s^{-1}) for 10 days to induce fruiting body differentiation (blue light) or maintained for 10 days in darkness (control).

2.2. RNA isolation, cDNA library preparation and Illumina sequencing

To obtain the expression changes of *P. eryngii* induced by blue light, two cDNA libraries were prepared including dark and blue light irradiated group. First, the total RNA extracted from pooled undifferentiated fruiting body (Fig. 1A) or differentiated fruiting body (Fig. 1B) obtained from 15 culture bags of each sample (blue light and dark control) was isolated using TRIZOL reagent (Takara, Japan) according to the manufacturer's protocol. The quality of the isolated RNA was detected by 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). For each group, to minimize biological variations, individual RNAs from three random samples were equally pooled for library construction and labeled as sample A. Sample B was prepared as sample A, and both samples(A and B) were processed separately.

The cDNA libraries were prepared using TruSeq RNA sample preparation kit from Illumina (San Diego, CA). Shortly, the mRNA was separated out using oligo(dT) beads from 5 μg total RNA. The RNAs were cut into 200 bp in fragmentation buffer. Then, mRNA was used to synthesize the cDNA and following end repair, A-base addition and ligation as Illumina's instruction. The short fragments with 200 read lengths were chosen for PCR amplification. Finally, the cDNA libraries were sequenced by Beijing Biomarker Technologies (Beijing, China) using Illumina HiSeq 2500 sequencer.

2.3. De novo assembly and annotation

The sequenced paired-end reads were first trimmed the adaptor sequences and the low quality bases were discarded. Subsequently, the clean data were used to conduct de novo assembly by Trinity (http://trinityrnaseq.sourceforge.net/) using the default parameters to obtain contigs. The contigs were connected to generate sequences which

Table 1
The reads and bases numbers for *P. eryngii* in blue light library and the dark control group.

Samples	Dark	Blue light
Base number	8,999,048,104	7,707,793,318
Clean reads	30,131,191	25,863,244
Mapped reads	25,089,384	21,060,706
Mapped ratio	83.27%	81.43%

extended on either end which called unigenes. The unigenes were then annotated using BLASTX programs by comparing to *P. eryngii* genome in JGI database (http://genome.jgi.doe.gov/), Swiss-Prot database (http://www.expasy.ch/sprot), Gene Ontology (GO) database (http://geneontology.org/page/go-database) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.genome.jp/kegg) with 1e-5 E-value cut off.

2.4. Analysis of differentially expressed genes

The differentially expressed genes (DEGs) between the two libraries were identified using the Fragments Per Kilobase of transcript per Million mapped reads (FPKM) method by DESeq R package (1.10.1) (http://bioconductor.org/packages/release/bioc/html/DESeq.html). The false discovery rate (FDR) was used to correct the p-value in multiple tests. If the FDR ≤ 0.001 and $|\log_2 FC| \geq 2$, the gene sets were determined as DEGs. After identification of DEGs, these genes were carried out into KEGG pathways analysis.

2.5. CAZymes annotation

The annotated unigenes from *P. eryngii* transcriptome that are related to carbohydrate-active enzymes were performed by the software CAZYmes Analysis Toolkit (CAT) (http://cricket.ornl.gov/cgi-bin/cat.cgi?tab=Home) [30], using the Carbohydrate Active Enzymes (CAZy) database (http://www.cazy.org) [31].

2.6. Quantitative real-time PCR validation of CAZymes

Six CAZymes and laccase with complete encoding regions and exhibiting significant expression levels were selected for validation by quantitative real-time PCR (qRT-PCR) method. Total RNA of the two samples including dark and blue light irradiated group was extracted by TRIZOL Plus RNA Purification Kit (Invitrogen). cDNAs were synthesized according to the manufacturer's protocol (Takara, Dalian, China). RT-PCR was performed on a CFX96 Real-Time System (BIO-RAD) with SYBR green as the fluorescent dye according to the manufacturer's protocol. Three technical and biological replicates were performed for all genes in each pool using GAPDH as the internal control gene, and the relative gene expression levels were calculated by the delta–delta Ct method.

2.7. Enzyme assay

The substrates colonized by mycelium were collected at primordial formation for studies of the enzymes activities. Extracellular enzymes were extracted as following: 10 g wet weight substrates were subject to 200 mL of 50 mM sodium acetate buffer (pH 4.8) and centrifuged at 180 rpm for 1 h in ice bath. Endo 1,4-beta glucanase, exo 1, 4-beta glucanase, β-glucosidase, pectin lyase, laccase and manganese peroxidase activities were determined as described [32–36].

2.8. Statistical analysis

All the experiments values presented in graphs and tables are mean ± SD calculated using Excel 2007. The enzyme activity results were the average results of 20 replicates, the RT-PCR experiments were conducted in triplicate. Multiple comparison tests were performed with *t*-test (significance levels = 0.05).

3. Results

3.1. Blue light induced primordium differentiated into fruiting body

The developmental process of *P. eryngii* can be divided into three main stages: the mycelia knot, primordium and fruiting body stages. The morphological changes under standard cultivation conditions were observed after mycelial maturation following the decrease in temperature and stimulation by blue light or in the dark. Fig. 1B showed that after blue light stimulation, the mycelium transformed from vegetative growth to reproductive growth, and the small fruiting body appeared in 10 days at the stage, while there was no differentiated fruiting body as determined by morphology (Fig. 1A). The results suggested that blue light can induce primordium differentiated into fruiting body.

3.2. Sequencing and gene annotation

The two cDNA libraries were sequenced by Illumina HiSeq 2500 system using paired-end (PE-150) method. In total, clean data were obtained after adaptor trimming and low quality reads were removed with every single read length of about 300 bp. For the two libraries, 30,131,191 and 25,863,244 clean reads were obtained from dark and blue light irradiation, respectively (Table 1). Among these clean reads, the Q30 (percentage of sequences with a sequencing error rate lower than 0.1%) was over 93% for both libraries. The clean reads retention rates of blue light and dark samples were 96.75% and 96.42%, respectively. The two libraries were combined into a single data set to perform de novo assembly. Using Trinity software, 118,145 transcripts and 32,876 unigenes were generated. The average length of unigenes was 887.52 bp with N50 length as 1904 bp (Table 2).

Meanwhile, RNA-Seq data was then compared to the *P. eryngii* reference genome posted on the JGI website using HISAT2 v2.05 software. The reference genome size was 44,606,964 bp, containing 609 scaffolds. The N50 value of the scaffolds is 241,626 bp, and the contigs value of N50 is about 87,948 bp. *P. eryngii* reference genome included 15,960 coding genes with function prediction. The results of RNA-Seq clean data matching to the reference genome are shown in Table 3. The threshold value of gene expression was counts ≥ = 10 per 1000 bp cDNA sequence, The gene expression of blue light and dark samples were 11,213 and 10,986, respectively. Among these genes, 10,856 genes were expressed in both samples (Fig. 2A). 11,343 genes expressed in at least one of the samples, accounting for 71.08% of the number of genes in the whole genome (Table S1). 357 genes only identified in blue light group (Table S2). The raw reads are available at the NCBI Sequence Read Archive (SRA) Database under Project Accession number SRP073348.

The amino acid sequences of unigenes were annotated using

Table 2
The assembled results for *P. eryngii* in blue light library and the dark control group.

Length range	Contig	Transcript	Unigene
200–300	9,979,772 (99.79%)	14,872 (12.59%)	13,373 (40.68%)
300–500	7079 (0.07%)	9089 (7.69%)	6360 (19.35%)
500–1000	5145 (0.05%)	13,548 (11.47%)	4645 (14.13%)
1000–2000	4553 (0.05%)	33,603 (28.44%)	4348 (13.23%)
2000 +	4011 (0.04%)	47,033 (39.81%)	4150 (12.62%)
Total number	10,000,560	118,145	32,876
Total length	396,541,557	229,794,548	29,178,108
N50 length	42	2845	1904
Mean length	39.65	1945.02	887.52

Table 3
The matched results of RNA-Seq Data to the *P. eryngii* genomes in blue light library and the dark control group.

	Dark	Blue light
Total reads	58,301,532 (100%)	49,874,850 (100%)
Total mapped	50,260,243 (86.21%)	43,498,022 (87.22%)
Fully match	28,993,893 (49.73%)	25,214,271 (50.56%)
1–2bp mismatch	1,5894,836 (27.26%)	13,694,745 (27.46%)
Single-position match	43,156,850 (74.02%)	3,7647,927 (75.49%)
Multi-position match	7,103,393 (12.18%)	5,850,095 (11.73%)
Unique match	49,492,504 (84.89%)	4,3067,799 (85.36%)
Unmatched	8,041,289 (13.79%)	6,376,428 (12.78%)

BLASTX against GO and KEGG databases with E-value of 1×10^{-5} cut off. Among them, 8854 (78.05%) were matched against public databases. In Table S1, the list of the annotated results analyzed from blasting against public databases was given. To further evaluate the function of the annotated genes, the sequences were assigned into KEGG classifications. A total amount of 8854 genes were divided into at least 120 functional groups. The top 10 KEGG pathways were showed in Fig.2B."Carbon metabolism", "biosynthesis of amino acids" and "oxidative phosphorylation" were represented as the largest group.

3.3. Identification and characterization of differentially expressed genes

To investigate the mRNA changes after irradiation by blue light, the DEGs were identified and annotated. A total of 539 unigenes were identified as DEGs, which contained 485 up-regulated unigenes and 54 down-regulated unigenes in blue light library compared to the dark control group (Table S3). Among these DEGs, several genes reported to take important roles in blue light response were up regulated, such as white collar 2 type of transcription factor gene (which was identified as blue light receptor), A mating type protein gene and MAP kinase gene. In addition, three genes of class I hydrophobin and pheromone receptor, which mediate interactions between the fungus and environment, were also identified as up regulated genes in blue light group. Besides, a series of genes in mitochondrial respiratory chain (cytochrome *b* subunit of succinate dehydrogenase, cytochrome *c*1,NADH-ubiquinone oxidoreductase, ubiquinol cytochrome-c reductase) were found upregulated, suggesting that oxidative phosphorylation may participate in blue light response process. Interestingly, genes associated with carbon, amino acids and fatty acid metabolism were also found regulated. Based on the above information, KEGG analysis was

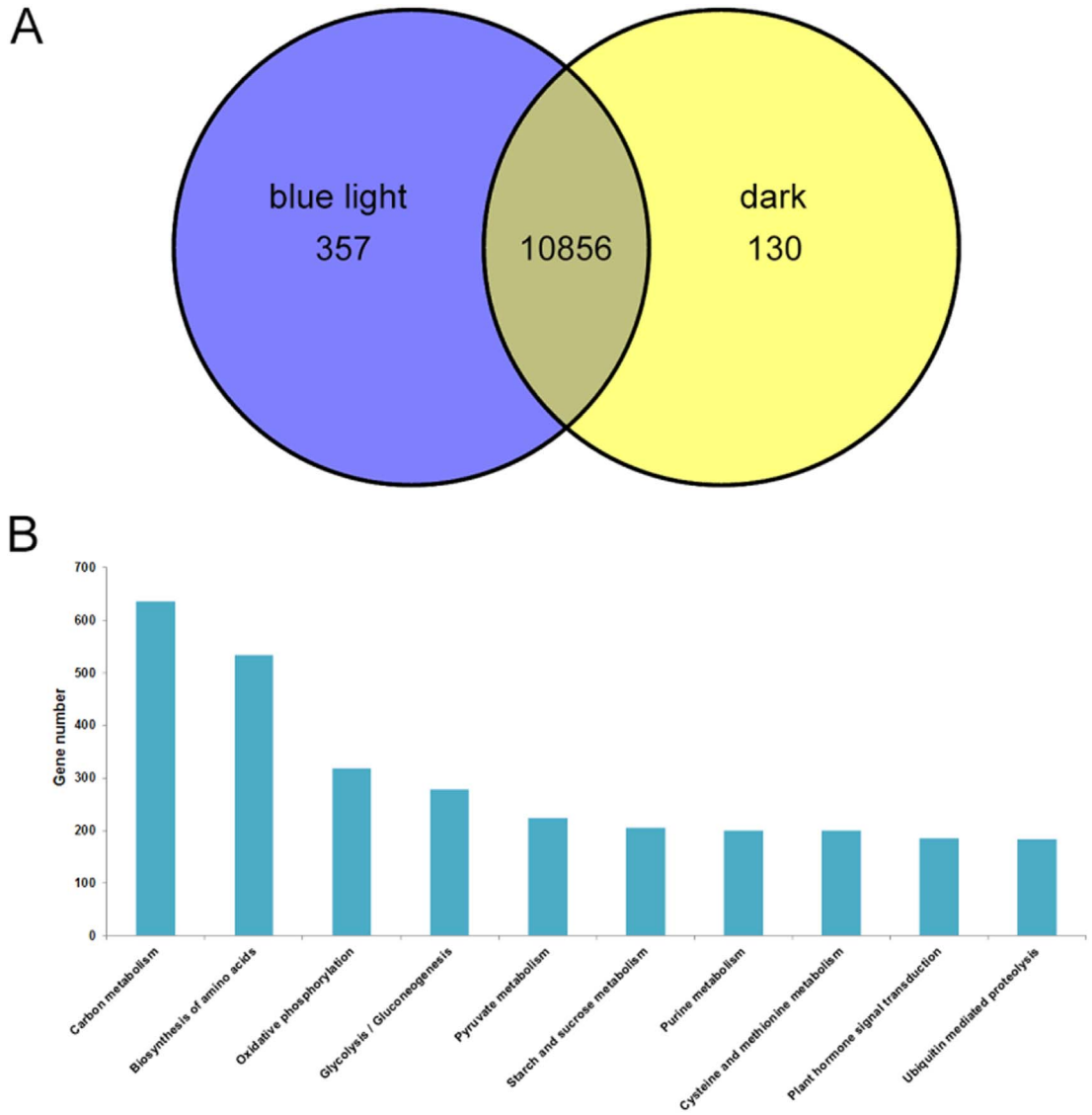

Fig. 2. The distribution for all of the identified genes and the top 10 KEGG pathways of these genes. A. A venn diagram of the identified genes distributed in blue light library and the dark control group. B. The top 10 KEGG pathways of all of the identified genes.

performed to classify the functions of annotated DEGs (Table S2). The top 10 KEGG pathways were showed in Fig. 3. We found that the DEGs were highly concentrated in “Carbon metabolism”, “Glycolysis/Gluconeogenesis” and “Biosynthesis of amino acids” pathways (Table S2 and Fig. 3), suggesting that primary metabolism pathways were the mainly pathways response to blue light during *P. eryngii* primordium differentiated into fruiting body.

3.4. Differentially expressed CAZymes genes in P. eryngii

CAZymes, including glycosyl hydrolases (GHs), carbohydrate esterases (CEs), polysaccharide lyases (PLs), carbohydrate-binding modules (CBMs), glycosyl transferases (GTs) and auxiliary activities (AAs) are involved in carbohydrate metabolism. In the present research, by searching the differentially expressed genes against the Carbohydrate-Active Enzyme database (CAZy), the BLASTx results indicated that 319 (59%) DEGs from *P. eryngii* transcriptome were identified as CAZymes. Among of these CAZymes, 187 GHs, 66 GTs, 19 CEs, 19 CBMs, 18 PLs and 10 AAs were identified (Table S4). Clear differences in the number and expression level of CAZymes genes from *P. eryngii* under blue light and dark were evident (Fig. 4), with blue light can induce a larger range of CAZymes, and more gene representatives per GHs, GTs, CEs, CBMs, PLs and AAs families.

3.5. Validation of differentially expressed CAZymes and laccase by qRT-PCR

To validate the DEGs with a biological replicate between blue light induced primordium and dark induced knots, the expression levels of six differentially expressed CAZymes (GH5(c12195), CBM13(c12920), 4CE(c16719), CBM 21(c7693), GH16(c18396), PL8(c11059)) and laccase 5 (c11608) obtained by RNA-seq analysis were validated by random selection for qRT-PCR analysis, with GAPDH serving as the reference gene. Results showed that gene expression profiling of these DEGs using qPCR revealed similar variation trends with RNA-Seq samples (Fig. 5).

3.6. Enzyme assay

In order to further investigate the effect of blue light on CAZymes activities, endo 1, 4-beta glucanase, exo1, 4-beta glucanase, β-glucosidase, pectin lyase, laccase and manganese peroxidase (MnP) activities were determined under dark and blue light stimulation. Fig. 6 showed that the blue light induced sample contained particularly high levels of cellulase (endo 1, 4-beta glucanase, exo1, 4-beta glucanase), with a 2.5-fold and 3-fold higher activity, respectively, compared with that of dark. In terms of hemicellulase and pectinase activities, the blue light stimulation sample furthermore displayed three fold higher levels of β-glucosidase and pectin lyase activities. Compared with dark, the activities of laccase were significantly higher than that in blue light stimulation, while MnP activities were lower. These results indicated that the activities of CAZymes were affected in primordium differentiated into fruiting body under blue light stimulation.

4. Discussion

Light is the key signaling element for fungi growth and development. Although both light quantity and quality are important for fungi life, the latter is most associated with fungal photomorphogenesis. Thus, in the mushroom farming industry, blue light is used to induce the growth of fruiting body [16,37,38]. Previous researches found and cloned a serial of blue light receptors such as WC-1/2, and these studies showed blue light was a key signaling component that regulates the gene expression and rearranges cellular metabolism globally. Our research also focused on searching for the important genes or pathways participated in blue light induced primordium differentiated into fruiting body of *P. eryngii*. Most importantly, three metabolic pathways were found regulated during fruiting body formation and growth by blue light irradiation in our research, including carbon metabolism, glycolysis/gluconeogenesis and biosynthesis of amino acids pathways. Interestingly, regulation of primary metabolic pathways in oyster mushroom mycelia induced by blue light stimulation were found in *Pleurotus ostreatus* [24], suggesting that metabolic pathways take important roles in response to the blue light signal.

The growth and development of *P. eryngii* is based on the nutrient of compost, which provides the available carbon sources [39]. The carbon metabolism and glycolysis/gluconeogenesis pathways mediate the polysaccharide decomposition and resynthesis in the mushrooms [40]. In the carbon metabolism pathway, CAZymes, especially cellulases and hemicellulases, are involved in the hydrolysis of plant cell wall polysaccharides, and play an important role in substrate degradation processes [41]. In the present research, 252 CAZyme genes in small fruiting

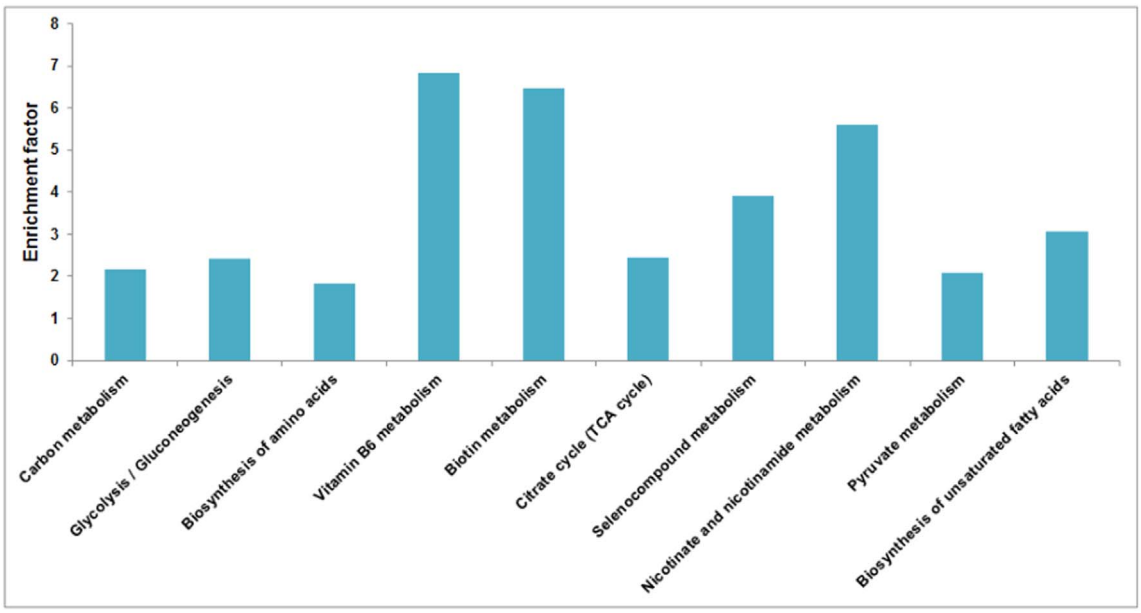

Fig. 3. The top 10 KEGG pathways of 539 differentially expressed genes.

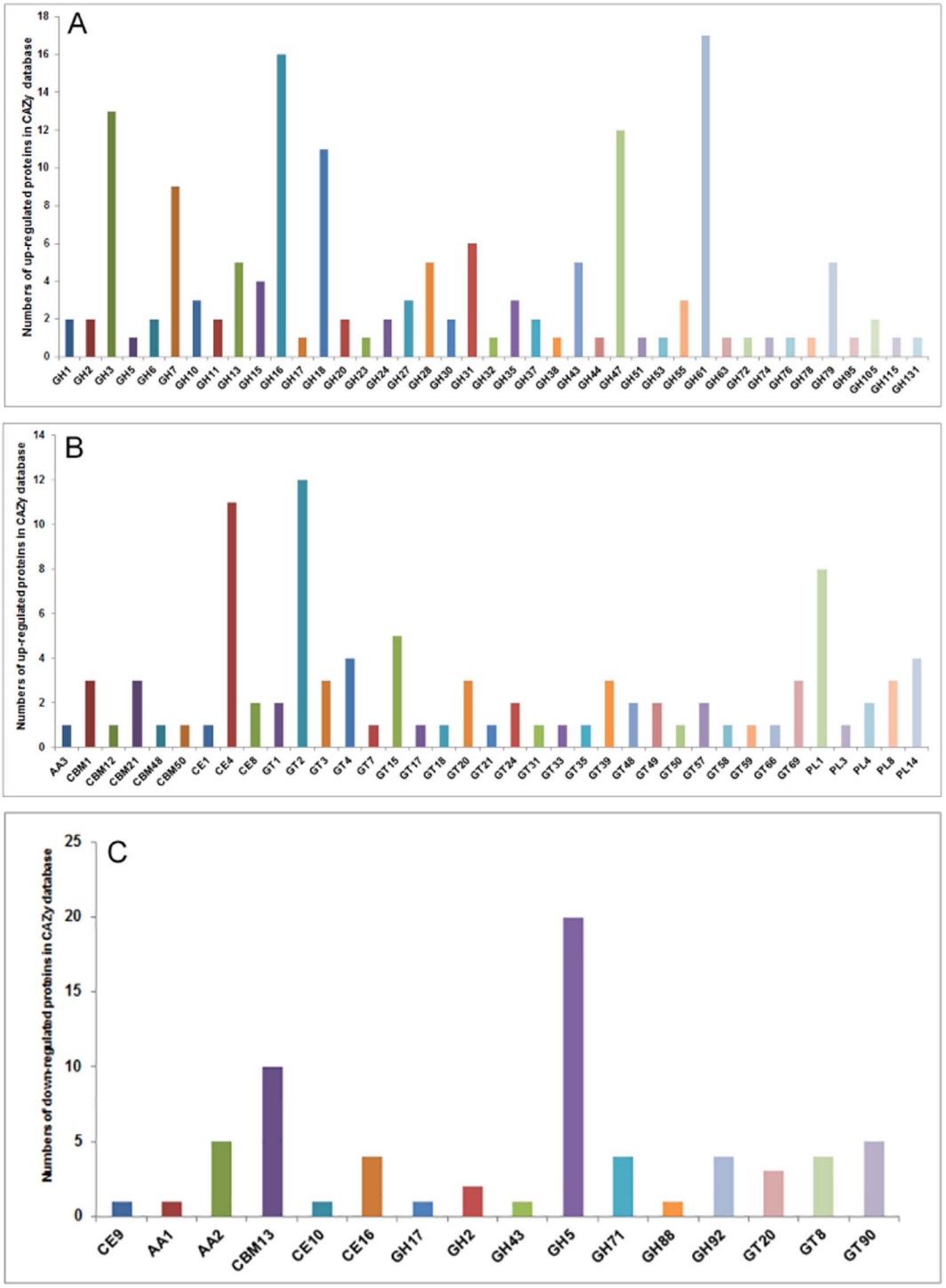

Fig. 4. Grouping and distribution analysis of differentially expressed CAZymes. A. Number and distribution of upregulated GHs in blue light library compared to the dark control group. B. Number and distribution of upregulated CEs, PLs, CBMs, GTs and AAs in blue light library compared to the dark control group. C. Number and distribution of downregulated CEs, PLs, CBMs, GTs and AAs in blue light library compared to the dark control group.

body induced by blue light displayed higher transcription levels than in undifferentiated fruiting body induced in dark, while 67 CAZyme genes in small fruiting body displayed lower transcription levels than in undifferentiated fruiting body. In general, the transcriptomic profile of CAZyme genes in two samples was similar. Enzyme activity results of the representative CAZymes indicated that the activities of these enzymes were affected in primordium differentiated into fruiting body under blue light stimulation. Among the CAZyme genes, GHs mainly hydrolyze the glycoside bond between carbohydrates or between a carbohydrate and a non-carbohydrate [42]. In our research, families GH16 (16 genes), GH3 (12 genes), GH 61 (17 genes), GH15 (4 genes), GH47 (12 genes), GH18 (11 genes) and GH28 (5 genes) were the dominant among these up-regulated CAZymes genes in primordium induced by blue light. GH16 family enzymes possess xyloglucanase activity and may degrade β-1,3-glucans or xyloglucans [15]. GH3 family enzymes shown that the enzymes that cleave β-hexosaminides are in fact retainingβ-glycoside phosphorylases [43,44]. GH 61 family enzymes were copper-dependent lytic polysaccharide monooxygenases

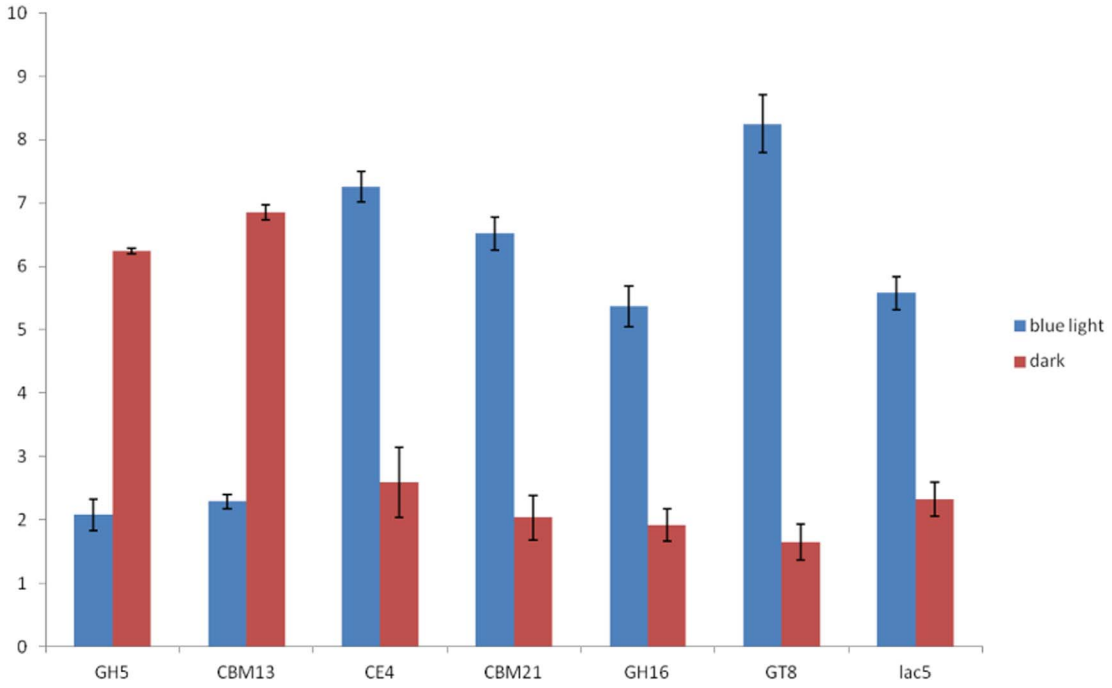

Fig. 5. Validation the expression levels of six differentially expressed CAZymes (GH5(c12195), CBM13(c12920), CE4(c16719), CBM 21(c7693), GH16(c18396), PL8(c11059) and lac5(c11608) by qRT-PCR. Error bars represent the mean ± standard deviation of triplicate experiments.

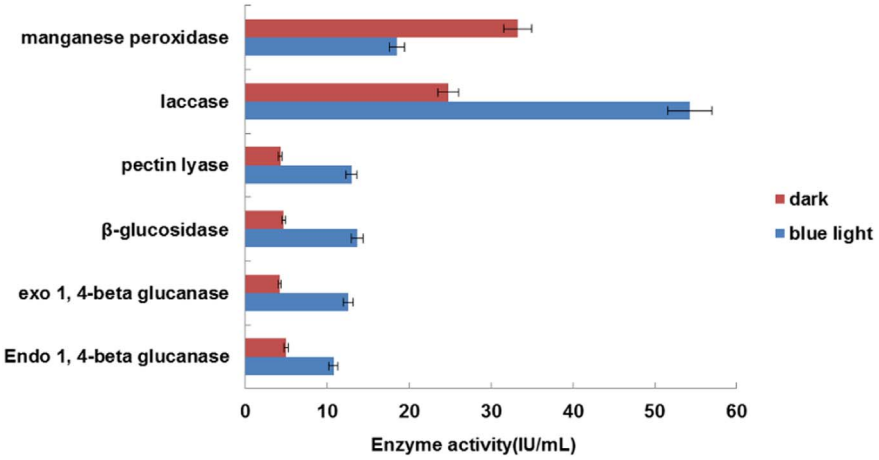

Fig. 6. Comparison of Endo 1,4-beta glucanase, exo 1,4-beta glucanase, β-glucosidase, pectin lyase, laccase and manganese peroxidase activities in primordium differentiated into fruiting bodies stage induced by blue light or dark. Error bars represent the mean ± standard deviation of triplicate experiments.

[45]. The functions of GH15 and GH 47 were not known in present. GH18 may degrade chitin and cleave the β-1,4linkage between *N*-acetylglucosamine residues at the base of the oligosaccharide chain while GH28 plays a critical role in pectin degradation [46,47]. Besides the upregulated GH genes, GH5 (20 genes) and GH71 (4 genes) were mainly shown down regulated in primordium induced by blue light. GH5 is one of the largest GH families and consists of a wide range of enzymes activity on different substrates, such as β-1,3-glucans, β-1,4-glucans in cellulose, and β-1,4-mannans in hemicelluloses [48,49]. The function of GH 71 was not known.

In GHs that degrade cellulose, hemicellulose, chitin, or arabinogalactan, the catalytic modules are often attached to one or more non-catalytic carbohydrate-binding modules (CBMs) [50]. CBMs function independently to assist carbohydrate-active enzymes. To date, 67 families of CBMs have been discovered [50]. In our research, families CBM 21 (3 genes) and CBM 1 (3 genes) were up-regulated in primordium induced by blue light. Family CBMs 21 contain approx. 100 amino acid residues, and some members have starch binding functions or glycogen-binding activities [51]. Family CBMs 1 are small proteins that consist of < 40 aa. Nearly all known CBM-bearing lignocellulose-degrading enzymes from fungi were CBMs 1 [52]. Family CBM 13 (10 genes), which has been structurally and biochemically analyzed for the first time within intact GHs (*Streptomyces* endo-1,4-xylanases) [53], was found down regulated in our study. It is also distributed in many carbohydrate-active enzymes, because it has evolved to acquire a variety of sugar-binding specificities.

Carbohydrate esterases (CEs) are enzymes catalyzing the deacylation of esters or amides in which sugars play the role of alcohol and amine [54]. They are currently classified in 16 different CE families, which show a great diversity in substrate specificity and structure [55]. In our research, families CE 4 (11 genes) and CE 8 (2 genes) were up-regulated in primordium induced by blue light, while CE16 (4 genes) and CE 9 (1 genes) were down regulated. Family CE 4 deacetylate polymeric carbohydrate substrates such as chitin, acetyl xylan and peptidoglycan [56]. Hemicellulolytic acetyl esterases classified in CE 16, it does not deacetylate polymeric substrates like acetyl glucuronoxylan but acted on acetylated xylobiose [57]. The functions of CE 8 and CE 9 were unclear.

The CAZy database (www.cazy.org) currently lists 103 different families for glycosyl transferases (GTs) [31]. Many of these enzymes currently have no known function and few have been biochemically characterized. In our research, GT 2 (11 genes)and GT 39 (3 genes) were up-regulated in primordium induced by blue light, while GT 22 (3 genes), GT 8 (4 genes) and GT 90 (5 genes) were down regulated.

P. eryngii degrade the lignin using oxidative enzymes classified as manganese peroxidases (MnP), versatile peroxidases (VP) and laccase. In addition to the auxiliary activities (AAs) genes including manganese peroxidase and aryl-alcohol oxidases (AAO), there are a number of accessory enzymes that participate in the process, including laccase, glyoxal oxidases (GLOX) and pyranose dehydrogenases and methanol oxidases. Laccase (7 genes) and manganese peroxidase (6 genes) were differentially expressed. These two genes take important part in lignin degradation [58]. Previous study has shown that laccase gene is involved in *Hypsizygus marmoreus* primordium initiation by increasing laccase activity [59]. In our research, laccase was also upregulated, suggesting that this enzyme plays important roles in primordium differentiated into fruiting body. Manganese peroxidase was down regulated, and the information about manganese peroxidase in primordium differentiated into fruiting body was limited. In addition, aryl-alcohol oxidase, DyP-type peroxidase and phenol oxidase were also identified as DGEs in our research. Besides, polysaccharide lyases (PLs) cleave a hexose-1,4-alpha- or beta-uronic acid sequence by beta-elimination. In our research, mainly PL 1 (8 genes), PL 14 (4 genes) and PL8 (3 genes) were up-regulated in small fruiting body induced by blue light. Similar to GTs, the functions of PLs were limited in present.

As described above, our research showed that most of the CAZymes were up regulated during primordium differentiated into fruiting body induced by blue light, suggesting that these enzymes play important roles in lignocellulose degradation to provide sufficient nutrition for *P. eryngii* primordium differentiated into fruiting body process. But the functions of these genes in primordium differentiated into fruiting body still need further research.

5. Conclusion

We have identified the *P. eryngii* transcriptome at primordium differentiated into fruiting body stage after blue light stimulation and dark using high-throughput sequencing approach, resulting in identifying 539 DEGs, including white collar 2 type of transcription factor gene, A mating type protein gene and MAP kinase gene, oxidative phosphorylation associated genes, CAZymes genes and other metabolism related genes. KEGG results showed that carbon metabolism, glycolysis/gluconeogenesis and biosynthesis of amino acids pathways were affected during blue light inducing primordium differentiated into fruiting body. 319 differentially expressed CAZymes genes including 187 GHs, 66 GTs, 19 CEs, 19 CBMs, 18 PLs and 10 AAs were identified. The expression pattern of seven representative CAZymes genes was further confirmed by qRT-PCR. Enzyme activity results indicated that the activities of CAZymes were affected in primordium differentiated into fruiting body under blue light stimulation. Characterization of these CAZymes genes will enhance our understanding on mechanism of *P. eryngii* primordium differentiated into fruiting body after blue light stimulation. Though being a necessary edible mushroom species in China, the molecular and genetic backgrounds maintains greatly uncharacterized. These data also provide a valuable resource for further and deeper studies on *P. eryngii*. Further studies should focus on the functional features of the CAZymes genes which might offer more in-depth knowledge of the mechanism and would aid mushroom farming in future.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2017.09.012.

Note: Total reads, all the number of reads in sample clean data: Total mapped, reads matched with genomes: Fully match, reads mapped with genemes, it is always matched: 1–2 bp mismatch, reads mapped with genemes, there are 1 to 2 bp of base mismatched: Single-position match, reads having only one mapped site in genomes: Multi-positon match, reads having no less than two matched sites in genomes: Unique match, reads having only one optimum-matched site in genomes: Unmatched, reads mapped with genemes, there is no matched site in genomes.

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